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BIOSENSORS for MARINE

and

OTHER ENVIRONMENTS

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INTRODUCTION

The purpose of this symposium, "Biosensors for Marine and Other Environments" is to bring together experts representing the fields of sensor development and sensor application to diverse biological systems. Although marine environments and bioreactors share many common features in their chemistry and biology, these areas are the domain of different scientific communities and it is not surprising that developments in monitoring are made in parallel rather than synergistically. We believe there is an important synergy and this symposium's purpose is to bring together the people active in both areas to explore the problems and solutions common to biotechnology and marine science. The symposium structure will be similar to that of a workshop creating an environment for cross disciplinary interactions. This format will encourage the sharing of new measurement techniques (and their use) to understand the state and dynamics of complex systems, which will lead to innovative ideas and ultimately a better understanding of the ocean and other natural environments.

Our ability to describe complex environmental systems is limited by our ability to measure their characteristics, especially as they change with time. The limitations associated with observing these characteristics are primary barriers to developing a better understanding of biological systems as small as the cell and as large as the marine ecosystem. However, due to the many common features of biological systems that do exist, the methods required to observe these two extreme systems are quite common.

The presentations at the symposium cover both techniques and applications. Throughout the one and one-half day meeting investigators will describe new advances in electrochemical, chemiluminescent, fluorescent and light-scattering techniques to characterize biochemical systems. Several presentations will also address the various biochemical processes that are being studied in the marine environment, the present limitations of the equipment used to study these marine processes and the harsh environment that future instruments must be designed for. The techniques to be discussed provide a unique opportunity to observe the state of biochemical systems at the microscale while the applications to be discussed will describe the observation needs of the marine scientist. These together will hopefully lead to greater collaborative (and synergistic) efforts between these two scientific communities resulting in a greater understanding of biochemical processes, whether they occur in a bioreactor or the ocean.

Professor Charles L. Cooney
Associate Director,
Biotechnology Process
Engineering Center

SYMPOSIUM AGENDA

Biosensors for Marine and Other Environments

April 3

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1:00-1:45	**Detection of Cytochrome P450 Induction as a Marker of Carcinogenic and Pollutant Contamination** John J. Stegeman, Woods Hole Oceanographic Institution
1:45-2:30	**Electrochemiluminescence: A Sensitive and Rapid Approach to Immunassays** Charles L. Cooney, MIT
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2:45-3:30	**Recombinant Firefly Luciferase as a Biosensor for Quantifying On-Line Viable Cell Mass Concentration** Daniel I. C. Wang, MIT
3:30-4:15	**Fiber Optic Sensors and Biosensors for Seawater Measurements and National Research Council Panel of Measurement Technologies for the Oceans** David R. Walt, Tufts University
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Biosensors for Marine and Other Environments

April 4

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9:30-10:15	**Microdrop Technology: A Solution to the Problem of Rapidly Determining Microbial Function, Composition and Number** James C. Weaver, MIT
10:15-10:30	**BREAK**
10:30-11:15	**Application of Light Scattering to the Determination of Cell Concentration in the Presence of Solid Substrates** Gregory Stephanopoulos, MIT
11:15-12:00	**Rapid Quantitation Method for DNA in Biological Samples** Jean-Francois Hamel, MIT
12:00-1:30	**LUNCH**
1:30-3:00	**Lab Tour**

SYNOPSES OF PRESENTATIONS

APRIL 3

9:00
Temporal, Spatial and Technological Considerations for the Use of Autonomous Instruments in the Oceanic and Coastal Environments

Dr. Craig D. Taylor, Woods Hole Oceanographic Institute

Oceanic margins are regions characterized by their high degree of spatial and temporal variability in biogeochemical properties. Heavily impacted by natural and anthropogenic inputs, variations in these properties can be of large amplitude, especially in coastal ponds and embayments that are positioned to intercept transport from land before entry into the ocean. Increasing concern for the effects on coastal ecosystems of increased nutrient loading through groundwater and streams and from direct waste inputs places a greater importance upon effective approaches for the study and monitoring of these environments.

In much the same way that sampling strategy in many oceanic studies has been controlled by ship availability rather than experimental need, labor intensive coastal environmental studies are nearly always heavily constrained by logistics and funding. The result is often a vastly undersampled research program in which the investigators are asked to predict the effect of an ongoing or proposed anthropogenic input or change in circulation against a high amplitude, variable background of natural phenomena. A typical seasonal study may consist of as few as four and up to 12 sampling periods per year within an environment that has ecosystem structuring periodic events of one week duration or less. Logistics often preclude investigating the potentially large effects of tide, diel cycle, and subtle stochastic events (such as successive cloudy days) upon the measured signal. Interpretation of data and realistic predictive assessment is made difficult when high frequency fluctuations approach seasonal signals.

A recurring recent theme in blue water and coastal biological oceanography relates to understanding the dynamics, mechanisms, and ultimately the predictability of the coupling between physical and biological processes. Interrelationships between biological activity and the physical and chemical processes that occur in the ocean are often complex. For example, events of major ecological significance may occur on a seasonal time scale or may result from sporadic and short-lived perturbations in the environment. Our strategy, therefore, has emphasized the need for a substantial increase in the temporal and spatial resolution of biologically relevant measurements, without at the same time sacrificing measurement duration. Proposed means for the gathering of necessary data emphasize, in addition to classic boat- and laboratory-based approaches, the use of strategically placed, unattended, moored instrumentation.

At present, investigators in the physical and bio-optical sciences have by-and-large been the most successful at combining long-term and high resolution measurements in the field. Unfortunately, most of the directly measured biological variables, such as primary production, that one may wish to use for correlation with chemical and physical data are not typically measured at a comparable temporal resolution. Often they are not even within the same one or two orders of magnitude. The primary reason is that biological measurements tend to require physical manipulation, which is labor intensive and often requires a laboratory setting.

Our laboratories have endeavored to reduce the temporal disparity between biological rate measurements, chemical measurements, and high resolution physical and biophysical

measurements. In particular we wish to assure that employed analysis rates are relevant to the time scale of environmental changes. One of our technical goals has been to make possible high resolution, long-term *in situ* measurements of primary production and other microbial rate processes on a cost-effective basis. A recently developed Submersible Incubation Device (SID), an autonomously functioning instrument that will conduct sequential incubation experiments directly *in situ* at user specified intervals, has shown that classically implemented seasonal primary production measurements may be undersampled 5-10 fold in many coastal ecosystem studies. In addition, autonomous instrumentation for *in situ* time series measurement of the major inorganic nutrients is in an advanced state of development and new commercial instrumentation has been applied to the long-term, high resolution measurement of oxygen in aquatic environments.

Technical aspects of these new developments will be considered and their application to studies in blue water and coastal oceanography discussed.

9:45
Biosensor Technology: Electrochemical Amperometric Approaches

Dr. I.J. Higgins, Cranfield Institute of Technology

Biosensors are usually comprised of a biological recognition element in close association with a physico-chemical transducer. In the presence of a specific analyte, an electrical signal is generated, the magnitude of which is proportional to analyte concentration. Many different transducers and formats have been demonstrated experimentally but only a modest number have been commercialized to date, mainly for medical diagnostic applications. Particularly notable products are glucose analyzers such as those produced by Yellow Springs Instrument Co. and other organizations, nerve-gas sensors such as the Thorn-EMI device and the pocket-sized diabetic self-monitoring instruments initially developed at Cranfield and later produced and marketed by Medisense Inc. These products and many others in development are amperometric electrochemical devices. A range of future products based on other transduction technologies, such as optical devices exploiting SPR principles are also in advanced development. Over the next few years rapid growth in biosensor products is expected, especially ones designed for the biomedical market.

Much of the high cost of R&D for novel biosensor configurations has already been met as a result of the major market opportunities in the biomedical area. The technologies are, in principle, applicable to the requirements of monitoring the marine environment. Clear targets and well-defined specifications are needed to minimize the time and expenditure required. This contribution will focus on opportunities to exploit advanced amperometric biosensor concepts.

Over the last 10 years, the Cranfield team have developed a range of novel approaches to amperometric systems, especially cheap, throw-away biosensors, aimed at medical applications. The Exac-Tech glucose pen was the first successful, commercial device based on this approach. Incorporation of a ferrocene derivative together with glucose oxidase in a printable carbon ink formulation permits the large-scale production of highly reproducible, cheap biosensors. This approach can be used for a range of other analytes and alternative mediators can be used to effect electron transfer between the enzyme redox center and the carbon conductor. Apart from ease of manufacture and enabling user-friendly formats, another major advantage of this system is the minimization of chemical interference. We have since extended for the technology to long-life, multi-use configurations for clinical analyzers, *in vivo* patient monitoring, for use in bioreactors and for meat quality assessment.

The mediated amperometic configuration also has considerable potential for affinity assays base on ELISA-type principles. This is possible using both antibody and nucleic acid based recognition systems. A cheap, portable, compact, immunodiagnostic biosensor is already at production prototype stage and could be used for measuring trace amounts of organic pollutants in seawater using appropriate labelled antibodies. Also of direct relevance to pollution of aquatic environments is a sensitive and specific method for bioelectro-chemical measurement of inorganic phosphate, which forms the basis of a simple amperometic biosensor. This employs two enzymes, nucleoside phosphorylase and xanthine oxidase and 7,7,8,8,-tetracyanoquinodimenthane (TCNQ) as mediator:

phosphate + inosine ——————→ hypoxanthine + ribose -l- phosphate - (l)

hypoxanthine + 2TCNQ + $2K^+$ ——————→ uric acid + 2KTCNQ - (2)

KTCNQ - e ——————→ TCNQ + K^+ -(3)

Reaction (3) is responsible for the anodic current generated by the biosensor.

Many organic pollutants of the marine environment have poor water solubility and are present in low concentrations. Biosensors can be configured to operate in organic solvents, allowing easy measurement of trace amounts of pollutants preconcentrated by extraction into an organic solvent. For example, an immobilized polyphenol oxidase-based biosensor has been used to monitor phenolic substances extracted into organic solvents such as chloroform.

In further examples, state-of-the-art biosensor technology based on interaction between electrochemical mediators and intact micro-organisms could readily be adapted to requirements for marine monitoring. We have recently completed the development of inexpensive portable, amperometric whole microorganism sensor for the dairy industry which measures total numbers down to 104 organisms per ml of milk in 20 minutes. This type of technology could be made more sensitive, especially for less chemically complex samples. Further sensitivity improvement could be achieved by pre-concentration or growth. There is also the possibility of making such devices organism specific.

In a related approach, biosensors for detecting trace amounts of herbicides in water have been demonstrated using chemical mediators and amperometric electrode systems to measure the reductive capacity of associated, illuminated immobilised microalgae. Herbicides inhibit photosynthetic electron transport leading to a reduction in current output of the device.

There are clearly many opportunities to adapt existing biosensor technology for applications in the marine environment, a few suggestions being made above. In some cases, devices could be developed rapidly by minor modifications of existing systems, in others more major development programs will be needed. It is obviously important to establish preferred performance specifications and formats at an early stage.

10:50
Polymer Membrane Based Electrochemical Ion Gas Sensors: Recent Advances and Future Prospects

Professor Mark E. Meyerhoff
Synopsis is not available at time of publication

11:35
Marine Environmental Measurement Needs

Dr. Paul D. Boehm, Arthur D. Little, Inc.

While great strides have been made in analytical methodologies, instrumentation, remote sensing techniques and sampling methods, the acquisition of marine environmental data needs improvement in several key areas: analytical measurements, combined sampling/analytical methods and environmental process determinations.

Analytical methodologies for many marine pollutants of concern remain fairly labor intensive due to a combination of the complexity of the matrix and the complexity of the analytical "soup" that characterizes all urban environments and most coastal areas. Methodologies presently lack chemical specificity at adequate sensitivities and also require removal of the analyte(s) from the matrix prior to analysis. Of greatest concern are the complex organic chemical pollutant classes such as the chlorinated hydrocarbons, polynuclear aromatic hydrocarbons, dioxins, organometallics, and others.

Sampling and combined sampling/analytical packages need to be representative of the environment. This sampling often involves sampling within the water column, sampling in the upper sediment column, and sampling of biological tissues to determine bioavailability (accumulation from water) and bioconcentration (accumulation from water plus food sources). While *in situ* measurements of conventional parameters such as dissolved oxygen are routinely made, at present sampling and analytical systems for organic and metallic pollutants are decoupled. There is a need to develop analyte-specific, combined sampling and analytical systems.

Of great interest to marine environmental scientists and regulators are process measurements. For example, the flux of pollutants from sediments to the bottom boundary layer is of great importance, as is the bioaccumulation potential of these pollutants. Many of these processes are driven by a combination of continuous and episodic events (e.g. storms). The mass transfer terms need to be determined and mass balanced need to be constructed from reliable representative measurements. Another set of processes that are presently thought to be important yet are largely unquantified are sea surface-atmospheric fluxes. These need to be determined for specific analytes on drainage basins emptying into coastal zone waters.

Perhaps the area of greatest potential for biosensors is in the development of techniques and monitoring tools that relate chemical fluxes and concentrations to biological effects. These effects can range from acute toxicity, to bioaccumulation, to sublethal stress. Biologists and toxicologists must have at their disposal a set of tools that can rapidly translate information on pollutant distributions to a spectrum of measurements that can give early warning signals regarding estuarine and coastal marine health.

1:00
Detection of Cytochrome P450 Induction as a Marker of Carcinogenic and Pollutant Contamination

Dr. John J. Stegeman, Woods Hole Oceanographic Institute

The introduction of specific immunological and molecular biological techniques has provided new understanding of the induction of cytochrome P-450 (monooxygenase or mixed-function oxidase catalysts) in fish, and its potential as a biomarker for xenobiotic exposure. This presentation

will describe our recent studies on the use of specific probes to identify and interpret the induction of cytochrome P-450 in marine fish. Analysis with polyclonal and monoclonal antibodies to specific forms of cytochrome P-450 has established relationships between polynuclear aromatic hydrocarbon (PAH) and polychlorinated biphenyl (PCB) inducible forms of cytochrome P-450 throughout the vertebrata. Teleost P-450 forms, e.g. P-450E from scup,
P-450LM4b from rainbow trout and P-450c from cod, are apparently homologous, and are orthologs of mammalian forms now classified with the P-450IA1 gene family. The patterns of mRNA transcription and translation during induction of teleost liver P-450E have been defined using cDNA and immunological probes. Catalytic assay and antibodies to P-450E have now been applied to analysis of environmental induction in field studies in Langesundsfjord (Oslo), San Francisco Bay, Narragansett Bay (Rhode Island), Bermuda and in the deep ocean. In several of these studies the levels of liver microsomal cytochrome P-450E detected in immunoblot were closely associated (r^2>0.9) levels of selected contaminant residues in the organisms or their environments.

The identity of specific compounds responsible for the induction are not yet known; studies are focusing on specific hydrocarbons and planar chlorinated hydrocarbons. The magnitude of induction depends on these specific structures, and on the status of biological (e.g. hormonal condition) and environmental (e.g. temperature) variables; the mechanism by which these factors influence induction is not yet known. The significance of P-450E induction to animal health depends on: (1) the catalytic capacities of P-450E, (2) the substrates presented to the organism, (3) the specific cells in liver or other organs in which the P-450 is induced and (4) the nature of coincident and consequent biological changes. Regardless of effects on animal health, P-450E induction is a distinct biomarker for exposure to persistent and potentially hazardous environmental chemicals.

1:45
Electrochemiluminescence: An Accurate and Rapid Approach to Immunoassay

Professor Charles L. Cooney, MIT

Immunoassays are highly specific for their analyte, and can be used to detect and quantify the analyte in a complex mixture, or to detect the presence of the analyte in a solution. The Electrochemiluminescence Immuno Assays (ECIA) utilize a marker molecule that will luminesce when, together with other components in solution, it is exited by an electric field. Binding to a solid phase modulates the signal from the marker. In an immunoassay the ECIA technique permits the use of a homogeneous format, eliminating the need for wash steps. We have used ECIA to monitor the production of monoclonal antibodies in bioreactors and have found that ECIA offers a fast, convenient and accurate method for this purpose.

2:45
Recombinant Firefly Luciferase As A Biosensor for Quantifying On-Line Variable Cell Mass Concentration

Professor Daniel I.C. Wang, MIT

Proper monitoring of bioreactors requires quantitative knowledge of the number or density of cells in the reactor. The ability to distinguish the viable cells from the non-viable cells is highly desirable. Current methods for viable cell determination involve removing a sample

from the bioreactor and visually counting stained cells or colonies under a microscope. This process is prone to error and is time-consuming. Improved methods are desirable to reduce time loss, reduce contamination risks and introduce on-line monitoring capabilities. We are therefore developing a novel, accurate, *in situ* method for determining viable cell mass concentrations.

The key component in this method is the enzyme, luciferase which is responsible for light emission in firefly tails. This enzyme uses ATP to drive the oxidative decarboxylization of L-luciferin. The cDNA for firefly luciferase has been successfully expressed in many cell types. In each case, the enzyme remains intracellular, in the cytoplasm or peroxisomes, and is fully active.

We hypothesize that the amount of luciferase expressed by each cell containing the recombinant gene will be the same, and thus the amount of light produced by each cell should also be the same. When such a culture is supplied with L-luciferin, light should be produced in proportion to the number of cells present. As non-viable cells cannot produce ATP, they should not emit any light. The rapid hydrolysis of any ATP released by lysed cells in the medium ensures that there will be no extracellular light production.

Experiments have been performed using a strain of *Escherichia coli* expressing the luciferase gene. Cultures of this recombinant *E. coli* have been grown to moderate cell densities: in excess of 12 g/l dry cell weight. These studies have confirmed that the light produced is directly proportional to the cell density. Furthermore, it was shown that no light is produced in the medium, nor was any produced by non-viable cells that were killed with heat.

In addition, a modular light-detection system has been assembled and used to monitor the process of *E. coli* fermentations via a fiber-optic link to the interior of the bioreactor. A mathematical model relating light production and cell densities is being developed. The bioreactor monitoring system will be used to evaluate that model, as well as to investigate the ability of this method to monitor the metabolic status of cultures.

3:30
Fiber Optic Sensors and Biosensors for Seawater Measurements

Professor David R. Walt, Tufts University

Optical sensors offer many advantages for oceanographic measurements including electromagnetic interference insensitivity, drift-free calibration, multiplexing, and *in situ* monitoring capability. A general overview of fiber optic sensor technology will be provided along with specific requirements and prospects for deployment of these sensors for seawater measurements. A CO_2 sensor has been developed along with a portable spectrometer. At-sea shipboard measurements were conducted during the 1989 JGOFS North Atlantic Bloom Experiment. Results of this study and improved sensor designs will be discused.

National Research Council Panel of New Measurement Technologies for the Oceans

In 1990, the Ocean Studies Board approved the formation of a panel to study promising technologies that could be applied to solving ocean measurement problems. The panel's mandate is to examine the state of presently available analytical technologies and to project potential developments in analytical instrumentation to the 5, 10 and 25 year time frames. A discussion of the framework of the panel's deliberations as well as an overview of various technologies will be provided.

APRIL 4

8:45
The Air-Sea Interface - Processes and Measurement Needs

Dr. Nelson M. Frew, Woods Hole Oceanographic Institution

Air-sea interactions encompass a wide range of physical, chemical and biological processes. Physical processes (insolation, momentum transfer) set the boundary conditions for subsequent chemical and biological processes such as nutrient distribution, gas exchange, seasonal changes in primary productivity, carbon cycling, etc. Additionally, feedback processes arise, e.g. exchange and uptake of carbon dioxide potentially alter atmospheric warming and subsequent climatological change. Studies of the interface between these two reservoirs, known as the marine microlayer, are thus of keen interest. This paper will broadly outline some of these processes, pertinent scientific questions and measurement needs. Discussion primarily will draw upon examples from my own research on biogenic sea-surface films, which influence or are influenced by these processes.

Biogenic films are an ubiquitous feature of the marine microlayer by virtue of the high free energy of a clean air-water interface. Organic materials produced at or near the interface and transported to the interface by diffusion or more active modes of transport (upwelling, vertical sheer, bubble scavenging, buoyant overturn) are readily adsorbed, in the extreme case forming marine slicks, i.e. areas of visibly damped wave motions. These chemical enrichment features, one monolayer thick, can readily be seen from space platforms because of their impact on surface roughness and emissivity across the EM spectrum. The physics of gas exchange is also affected due to changes in near-surface turbulence. In addition, biogenic films affect the size, distribution and composition of aerosols (via sea spray and bursting bubbles). A dynamic interplay of factors including biological production, intensity of physical transport processes, diffusive exchange, microbial uptake and transformation and photochemical transformations controls the abundance and properties of these films.

Attempts to understand slick formation, distribution and dissipation (lifetimes) lead quickly to basic questions that are difficult to address using current techniques. Knowledge of the sources of surface-active organic material is of prime importance since composition determines interfacial viscoelastic properties and absorption/reflection of radiation. What are the supply terms? Does the supply region extend to the pycnocline due to vertical water motions or migration of an organisms (diel cycle)? Is the microlayer a distinct habitat for marine neuston? Are there specific assemblages of organisms unique to the microlayer? What specific adaptations allow organisms to exploit this high stress environment (high UV, salinity extremes)? What biomarkers (chemical signatures) might be useful in identifying and tracking organisms? What are the loss terms that balance supply: redissolution, partitioning to surfaces, photooxidative losses? What are the integrated fluxes to the interface and how large are turnover rates for thermal, microbial and photochemical transformations in the microlayer itself? To what extent do film properties undergo hysteresis or 'age' due to these processes? Is the microlayer a significant source of gases and what do near-surface gradients look like?

General measurement needs include the ability to measure small quantities of material in dilute solution with a high degree of specificity in the presence of interferences and high salt concentrations. Specific needs range from sensing of simple molecules (metabolic products, gases) to recognition of complex structural moieties. Can biosensors be developed to measure extracellular enzymatic activity, for example, or to detect specific exudates such as stress related products or protective pigments? Given microlayer particulate enrichments, distinction of living cells from particulate detritus and assessment of taxonomy and physiological state of organisms becomes important. There is a particular need for improved

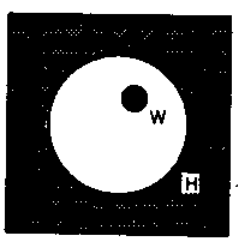
means of microlayer sampling, perhaps using selective affinity and transfer techniques. Sensor technology could also make a significant impact if ways of assigning timescales to some of the processes mentioned here could be developed. Sensor design considerations should place emphasis on flow-stream or preferably, *in-situ* measurements, low unit cost, minimal power consumption and compatibility with telemetric data transmission so that large-scale synoptic studies using towed platforms or drifters are feasible. The potential role of biosensor technology in answering some of these questions remains to be fully defined, but it is clear that any contributions along these lines will be useful not only to air-sea process studies, but also to studies of broader oceanic phenomena, whether in the upper ocean or the benthic regime.

9:30
Microdrop Technology: A Solution to the Problem of Rapidly Determining Microbial Function, Composition and Number

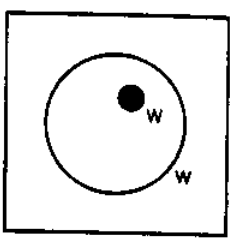
Dr. James C. Weaver, MIT

Microdrop Technology combines GMDs (gel microdroplets) and optical measurements to provide a new solution to bioanalytical problems. In the case of microorganisms, this includes counting cells, identifying cells, and determining their susceptibility to various molecules, even for mixed populations. Both "classic" biotechnology (optical and chemical indicator measurements of growth and activity) and "new" biotechnology (immunoassays and gene probes) can be used together with GMDs.

Microdrop Technology rapidly and gently converts cell preparations into a large number of GMDs, which are typically 10 to 100um diameter (e.g. agarose) particles. Poisson statistics governs the presence of cells, with the result that most occupied GMDs initially contain one cell (strictly, one colony forming unit). The two general versions shown below can be used with individual cells or individual microcolonies:



"Surround version"
(essentially a microminiaturized microtitier well)



"Open version"
(essentially a microminiaturized petri dish)

SURROUNDED VERSION: The circle represents a GMD, the solid dot an initial single cell, and "W" denotes "water" or aqueous phase. A permeability barrier is provided by surrounding GMDs with an inert hydrocarbon fluid ("H") which causes most water soluble molecules to be retained. OPEN VERSION: GMD in open communication to the surrounding aqueous medium. No permeability barrier is provided, so that molecular transport through the porous gel matrix readily occurs. This allows changes in chemical concentrations of the external medium to be rapidly communicated to the cell(s) within the GMDs. This version allows microcolonies of two

or more cells to form, but with orders of magnitude better ability to change incubation or test conditions. Provision of "capture sites" within the gel matrix allows protein secretion of individual cells and microcolonies to be determined.

Microdrop Technology provides assays based on function such as clonal growth, biochemical activity and molecular secretion (and the effects of compounds on these functions) of individual cells within a cell population. This provides a microassay system that combines optical measurements (e.g. ordinary microscopy, flow cytometry, inexpensive image analysis) with flexible cell culture conditions.

Other important attributes are: (I) GMDs are physically manipulable and can be handled much like cells (e.g. suspended, pipetted, centrifuged), and (2) GMDs rapidly exchange molecules with the external medium by diffusion, which allows rapid changes in the exposure of individual cells and microcolonies within GMDs to many different incubation conditions. Microdrop Technology has been demonstrated using mammalian, yeast and bacterial cells.

10:30
Application of Light Scattering to the Determination of Cell Concentration in the Presence of Solid Substrates

Professor Gregory Stephanopoulos, MIT

The determination of cell concentration is of fundamental importance in most biotechnological processes. However, this measurement is often obscured by other solid particles that are present along with cells in the biological environment and serve as nutrient sources for cell growth. This work investigated the light scatter properties of cell and solid substrate mixtures with the aim of developing a light scatter-based sensor for biomass in the presence of solid substrates.

Two crucial observations were made. First, that the light scatter from cells is a near linear function of cell concentration. Second, that invariant regions exist in light scatter spectrum in which the light scatter reading is independent of solid substrate concentration and a function only of cell concentration. The interactions of scatter and absorbance were described in a model reflecting the hypothesis that invariant regions are caused by changes in the absorbance of the solid substrate as a function of wavelength. Furthermore, an algorithm was developed for the utilization of light scatter data in the determination of biomass in cell-solid substrate mixtures.

This presentation will review our findings on light scatter in mixtures of *Bacillus subtilis* cells and typical solid substrates for fermentations. Examples of biomass estimation in complex fermentation media will be presented utilizing the presence of invariant regions in the light scatter spectrum, and so obtained biomass estimates will be compared to those typically available from DNA and carbon dioxide evolution measurements. The agreement is generally good indicating that the proposed technique is a valid method for the determination of biomass in industrial fermentation media. The availability of reliable on-line biomass measurements removed the most difficult obstacle in the development of advanced systems for fermentation diagnosis and control.

11:15
Rapid Quantitation Method for DNA in Biological Samples

Dr. Jean-Francois Hamel, MIT

This presentation will show how DNA can be quantified down to picogram levels using a powerful technique based on a light-addressable potentiometric sensor suitable for a wide variety of biological systems. The Threshold™ system can be employed in many process conditions, and its use has become widespread in both the pharmaceutical and the biotechnology industries. Several examples that describe the measurement of contaminating DNA in monoclonal antibodies, will be presented. Furthermore, we will examine the impact of this sensor-based technology on process development and on process monitoring for the removal of contaminating DNA with concomitant purification of monoclonal antibodies and other proteins.

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A novel chemical sensor has been developed in which the polymer ethylene-vinyl acetate is used as a controlled-release system to deliver reagents to the sensing region of an optical fiber for a homogeneous competitive immunoassay based on fluorescence energy transfer. A competition reaction is used to enable continuous measurements of the solution antigen concentration. More generally, the technique allows irreversible indicating chemistries to be used in the construction of chemical sensors that can measure continuously for long periods. Although the sensor configuration has not been optimized in all respects, data are presented for a model system in which a flourescein-labeled antibody and Texas Red-labeled immunoglobulin G (IgG) are used.

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Downs, M.E.A., Warner, P.J., Turner, A. P.F. & Fothergill, J.C., **Optical and Electrochemical Detection of DNA**, Biomaterials, Vol. 9, January 1988.

There is a growing demand for the production of a DNA biosensor with applications in medicine, the food industry, agriculture, veterinary science and environmental science. In this paper we describe methods for the optical and electrochemical detection of DNA using the enzyme horseradish peroxidase (EC I.II.I.7) and glucose oxidase (EC I.I.3.4). We have used bis-methylacridinium nitrate and luminol for the optical detection of DNA using a purpose built, inexpensive luminometer. Using this system detection limits of 10^{-11} g of plasmid DNA have been observed. Electrochemical detection of DNA was carried out by the use of a fluoride ion selective electrode and stripping voltametry. DNA was detected down to 10^{-9}-10^{-10} g of DNA by the enzymatic release of halogen ions from organohalogen compounds.

Frew, N M., Goldman, J. C., Dennett, M. R., & Johnson, A. S., **Impact of Phytoplankton-Generated Surfactants on Air-Sea Gas Exchange**, Journal of Geophysical Research, Vol. 95, No. C3, March 1990, pp. 3337-3352.

The effect of surface-active organic matter generated by seven common species of marine phytoplankton on gas exchange rates under turbulent conditions at the air-water interface was determined. Reductions in oxygen evasion rates ranging from 5 to 50% were observed relative to clean seawater controls. Relative oxygen exchange coefficients (expressed as $R=K_w$ (sample K_w (control)) were shown to be sensitive to small changes in total dissolved carbohydrate at concentrations < I mgC (carbon) L^{-1} and to asymptotically decrease to a lower limit (R=55-70%) at concentrations between 2 and 6 mgC L^{-1}. A corresponding relationship was observed in which R decreased with increasing relative surfactant amounts derived from surface pressure-area measurements. However, gas exchange reductions were significant for plankton exudate samples displaying surface pressures <I mN m^{-1}. It thus seems that condensed monolayer films are not a prerequisite for reduced gas exchange and that relatively soluble surfactants derived form phytoplankton can strongly affect the dissipation of near-surface turbulence and lead to changes in the Schmidt number dependency of K_w.

Frew, N.M., Johnson, C.G. & Bromund, R.H., **Supercritical Fluid-Mass Spectrometry of Carotenoid Pigments**, In: "Supercritical Fluid Extraction and Chromatography - Techniques and Applications," B. Carpenter and M. Sevenants, (Eds.), ACS Symposium Series, 366, 1988, pp.208-228.

Goldman, J C., Dennett, M.R. & Frew, N.M., **Surfactant Effects on Air-Sea Gas Exchange Under Turbulent Conditions**, Deep-Sea Research, Vol. 35, No. 12, 1988, pp.1953-1970.

In a series of laboratory gas exchange studies we found that under turbulent conditions additions of two synthetic surfactants (polyethylene oxide and oleyl alcohol) to distilled water and seawater led to reductions in oxygen evasion at the air-liquid interface. The oxygen exchange coefficient relative to that of a distilled water control asymptotically reached a lower limit of ~50% as surfactant concentration was increased. For natural seawater samples, an asymptotic reduction in relative gas exchange rate was demonstrated for increasing amounts of surface-active material as determined from surface pressure-area isotherms. Possibly surfactants act to reduce gas exchange by creating surface pressure forces that oppose and reduce turbulent eddy velocities and, correspondingly, reduce surface renewal. However, the greatest reductions in the oxygen exchange coefficient occurred at initial surface pressures (π_i) less than 0.5 mN m^{-1}. This result may have been due to the presence of soluble surfactants, which are known to be very effective in reducing gas exchange but which do not display concentration-dependent surface pressures.

Gordon, N.F., Tsujimura H. & Cooney C.L., **Optimization and Simulation of Continuous Affinity-recycle Extraction (CARE)**, Bioseparations, 1, 1990, pp.9-21.

Goyet, C., Walt, D.R. & Brewer, P.G., **Development of a Fiber Optic Sensor for Measurement of pCO_2 in Seawater: Design Criteria and Sea Trials**, submitted Deep Sea Research.

Haasch, M.L., Wejksnora, P.J., Stegeman, J.J. & Lech, J.J., **Cloned Rainbow Trout Liver P|450 Complementary NA as a Potential Environmental Monitor**, Toxicology & Applied Pharmacology, 98, pp.362-368.

Hafeman, D.J., Parce, W.J. & McConnell, H.M., **Light-Addressable Potentiometric Sensor for Biochemical Systems**, Science, Vol. 240, May 1988, pp.1182-1185.

Hamel, J.F. & Cooney, C.L., **Recovery of an Intracellular Enzyme from an E. *coli* Homogenate using Submicron-Sized Polymeric Particles: Small-Scale Evaluation and Pilot-Plant Design**, Proceedings of the 4th Symposium on Protein Purification Technologies, Clermont-Ferrant, France, March 1990.

Hardy, J.T., Crecelius, E.A., Antrim, L.D., Kresser, S.L., Broadhurst, V.L., Boehm, P.D., Steinhauer, W.G. & Coogan, T.H., **Aquatic Surface Microlayer Contamination in Chesapeake Bay**, Marine Chemistry, 28, pp.333-352.

Hendry, S.P., Higgins, I.J. & Bannister, J.V., **Amperometric Biosensors**, Journal of Biotechnology, no. 15, 1990, pp.229-238.

Electrochemical, optical piezoelectric and calorimetric routes linking biology to electronics have demonstrated the basis for a range fo biosensors (Turner et al., 1987a). A biosensor is therefore defined as an analytic device incorporating a biological or biologically devised sensing element either intimately associated with or integrated within a physiochemical transducer. Biosensors are distinguished from bioprobes because the latter are sensors used for *in vivo* monitoring (Thompson and Krull, 1986).

The diversity of biological elements incorporated into biosensors has expanded steadily since the pioneering work of Clark (1987) leading to the construction of enzyme electrodes. Intact microorganisms (Karube, 1987), tissues (Sidewell and Rechnitz, 1986) and antibodies (Kress-Rogers and Turner, 1988) are increasingly being reported in the literature on a biosensor configuration. Recently, attention has also focussed on the use of DNA probes for the detection of human genetic disorders and pathogens. Enzyme labels such as horseradish peroxidase, alkaline phosphatase and glucose oxidase which are commonly used for immunoassays have also been used for the electrochemical detection of DNA-DNA hybridization (Downs et al., 1987).

Higgins, I.J., Swain, A. & Turner, A.P.F., **Principles and Application of Biosensors in Microbiology**, Journal of Applied Bacteriology Symposium Supplement, 1987, pp.935-1049.

Biosensors exploit biological recognition systems by coupling such sensing elements with suitable transducers, enabling the conversion of analyte concentration or biological activities into digital electronic signals. The microbiologist has two major interests in the field of biosensors: (I) Many sensing elements are derived from microbiological sources, such as whole bacterial cells, microbial enzymes and DNA sequences, underlining the necessity for a significant input by the microbiologist into the development of biosensors.

(2) The microbiologist will almost certainly be a principal end-user of biosensors for both laboratory-based procedures and for wider applications in industrial, environmental and clinical areas. The purpose of this contribution is to review the field of biosensors specifically from the point of view of the microbiologist, both as a biosensor developer and as an end-user.

Kennedy, M.J., Thakur, M.S., Wang, D.I.C. & Stephanopoulos, G., **Techniques for the Estimation of Cell Concentration in the Presence of Solid Particles: A Review**, Biotechnology Advances, (submitted) (1991).

Kennedy, M.J., Wang, D.I.C. & Stephanopoulos, G., **Estimating Cell Concentration in the Presence of Solid Substrate: Light Scatter Observations**, Biotechnology and Bioengineering, (submitted) (1991).

Kennedy, M.J., Thakur, M.S., Wang, D.I.C. & Stephanopoulos, G., **Estimating Cell Concentration in the Presence of Solid Substrate: Estimation and Performance**, Biotechnology and Bioengineering, (submitted) (1991).

Kulp, T., Camins, I., Angel, S.M., Munkhom, C. & Walt, D.R., **Polymer Immobilized Enzyme Optrodes for the Detection of Penicillin**, Analytical Chemistry, 59, 1987, pp.2849.

Kung, V.T., Panfili, P.R., Sheldon, E.L., King, R.S., Nagainis, P.A., Gomez Jr., B., Ross, D.A., Briggs, J. & Zuk, R.F., **Picogram Quantitation of Total DNA Using DNA-Binding Proteins in a Silicon Sensor-Based System**, Analytical Biochemistry, 187, 1990, pp.220-227.

We report a rapid and reproducible method to quantify total DNA at picogram levels. Two high-affinity DNA-binding proteins are used to construct a sandwich assay and a semiconductor sensor is used for quantitation. Single-stranded DNA-binding protein (SSB) from *Escherichia coli* is conjugated with a linker molecule, biotin, for specific capture of the DNA complex onto a membrane. Monoclonal, anti-DNA antibody is conjugated with an enzyme, urease, for signal generation. To detect DNA, a sample is denatured to form single-stranded DNA and then incubated with a reagent containing both DNA-binding protein conjugates and streptavidin. After incubation of the reagent with the DNA sample for 1 h at 37 degrees C to form a complex of streptavidin-biotin-SSB-DNA-anti-DNA-urease, the mixture is filtered through a biotin-coated nitrocellulose membrane which binds the streptavidin component of the complex. The unbound reagent is washed off the membrane, and then the captured DNA complex is detected with a light-addressable potentiometric sensor which measures the pH change catalyzed by the urease in the complex. This assay can detect 2 pg of DNA with a quantitation coefficient of variation of less than 10% in the range 10 to 200pg.

Lee, I.H. & Meyerhoff, M.E., **Enzyme-Linked Flow-Injection Immunoassay Using Immobilized Secondary Antibodies**, Mikrochim. Acta. III, 1988, pp.207-221.

Lohrenz, S.E., Taylor, C.D. & Howes, B.L., **Primary Production of Protein. II. Algal Protein Metabolism and its Relationship to the Composition of Particulate Organic Matter in the Surface Mixed Layer of Salt Pond, MA.**, Marine Ecology Program Series, 40, 1987, pp.175-183.

Luo, S. & Walt, D.R., **Fiber Optic Sensors Based on Reagent Delivery with Controlled Release Polymers**, Analytical Chemistry, 61, 1989, pp.174.

Luo, S. & Walt, D.R., **Avidin-Biotin Coupling as a General Method for Preparing Enzyme-Based Fiber Optic Sensors**, Analytical Chemistry, 61, 1989, pp.1069-72.

McAullife, C.D., Boehm, P.D., Foster, J.C., Overton, E.B. & Page, D.S, **Monitoring Chemical Fate of Spilled Oil**, Oil Spill Studies: Measurement of Environmental Effects and Recovery, J.R. Gould (Ed.), American Petroleum Institute, Washington, D.C., 1988, pp.18-56.

McNeil, C.J., Higgins, I.J. & Bannister, J.V., **Amperometric Determination of Alkaline Phosphatase Activity: Application to Enzyme Immunoassay**, Biosensors 3, 1987/88, pp.199-209.

An amperometric assay for alkaline phosphatase has been developed using a novel substrate (N-ferrecenoyl)-4-aminophenyl phosphate. In the presence of alkaline phosphatase the substrate is converted to (N-ferrocenoyl) -4- aminophenol which shows an oxidation peak at + 180 mV. The change in peak current at + 180 mV was found to be related to the enzyme concentration. The assay was found to be suitable for enzyme linked immunoassay using alkaline phosphatase as the marker enzyme.

Meyerhoff, M.E., Park, S.B., Yim, H.S. & Cha, G.S., **Anion-Selective Polymeric Membrane Electrodes: Progress and Challenges**, Proceedings of Symposium on Methodology and Clinical Applications of Electrochemical and Fiber Optic Sensors, AACC, Washington, 1990, pp.65-88.

Powell, K.T. & Weaver, J.C., **Gel Microdroplets and Flow Cytometry: Rapid Determination of Antibody Secretion by Individual Cells Within a Cell Population**, Biotechnology, Vol. 8, 1990, pp.333.

We report a new method capable of rapidly determining the secretion of biologically important macromolecules from each of many individual cells within a large population. This method combines flow cytometry with gel microdroplets (GMDs), which in this study were agarose particles ranging from about 53 to 88 um in diameter. The GMDs were formed from a liquid 2.5% agarose suspension with cells at a concentration which yielded mostly zero or one cell per GMD. A large number of extracellular binding sites were also provided within each GMD, allowing the capture of secreted molecules, and their subsequent measurement by solid phase, fluorescence immunoassay. The method was explored using a model system of mouse hybridoma (secreting) and mouse masticytoma (non-secreting) cells. The method was able to determine subpopulations of individual cells that secreted antibody in less than fifteen hours after receipt of a conventional cell suspension.

Rawson, D. M., Willmer, A. J. & Turner, A. P.F., **Whole-Cell Biosensors for Environmental Monitoring**, Biosensors, 4, 1989, pp. 299-311.

Concern over the pollution risk to drinking water from industry and agriculture is growing, and the need for continuous on-line monitoring recognized. There is increasing use of living

organisms as the sensitive agent to detect the presence of pollutants, and whole-cell biosensors are seen to have particular advantages in such environmental monitoring. The development of a mediated amperometric biosensor, incorporating the cyanobacterium Synechococus as the biocatalyst, for on-line herbicide monitoring is described. The biosensor is able to detect a wide range of herbicides with sites of action on the photosynthetic electron transport chain, at concentrations down to 20 ug litre $^{-1}$ and possesses a working life of up to 7 days. The use of alginate immobilisation of the biocatalyst to overcome the problems associated with obtaining a realistic shelf life for the biosensor is discussed.

Repeta, D.J. & Frew, N.M., **Carotenoid Dehydrates in Recent Marine Sediments. The Structure and Synthesis of Fucoxanthin Dehydrate,** Organic Geochemistry, 12, 1988, pp.469-477.

Sauer. T.C., Durrell, G.S., Brown, J.S., Redford, D. & Boehm, P.D., **Concentrations of Chlorinated Pesticides and PCBd in Microlayer and Seawater Samples Collected in Open-Ocean Waters off the U.S. East Coast and in the Gulf of Mexico 1989,** Applied Science, Elsevier, pp.235-257.

Stegeman, J.J., Kloepper-Sams, P.J. & Farrington, J.W., **Monooxygenase Induction and Chlorobiphenyls in the Deep Sea Fish *Coryphaenoides armatus*,** Science, 231, pp.1287-1289.

Stegeman, J.J., **Cytochrome P450 Forms in Fish: Catalytic, Immunological and Sequence Similarities,** Xenobiotica, 19, pp.1093-1110.

Taylor, C.D. & Doherty, K.W., **Submersible Incubation Device (SID), Automonomous Instrumentation for the In situ Measurement of Primary Production and Other Microbial Rate Processes,** Deep-Sea Research, 37, 1990, pp.343-358.

Taylor, C.D. & Howes, B.L., **Role of High Frequency Sampling in Studies of Primary Production and Oxygen Status in Coastal Ecosystems,** Nature, (submitted).

Trojanowicz, M., Pobozy, E. & Meyerhoff, M.E., **Direct and Replacement Ion Chromatography With Potentiometric Detection Using Bromide Sensitive Electrode,** Analytical Chemistry, 1989, pp.109-119.

Turner, A.P.F., Ramsay, G. & Higgins, I.J., **Applications of Electron Transfer Between Biological Systems and Electrodes,** Proceedings of Industrial and Medical Applications of Bioelectrochemistry, I.J. Higgins (Ed.), Cranfield Biotechnology Centre, September, 1982.

The demonstration of rapid and reversible electron transfer between cytochrome c and a gold electrode in the presence of 4,4-bipyridyl (Eddowes & Hill, 1977; Albery et al., 1981) offered the opportunity for development of a new generation of bioelectrochemical devices. Extension of this work on 'promoted' electron transfer to other redox proteins such as bacterial ferredoxin (Armstrong et al., 1982), together with successes in coupling oxidoreductases such

as nitrate reductase to these modified electrodes (Hill et al., 1981), provided a means of tightly linking enzyme-catalysed reactions to solid electrodes. A complementary method of achieving direct electron transfer is the use of chemical 'mediators', which, unlike the 'promoters' they replace, undergo redox reactions during translocation of electrons between proteins and electrodes (Plotkin et al., 1981; Turner et al., 1982). Mediators may also be immobilized on or within the electrode surface, which when coated with an appropriate oxidoreductase provide commerically important catalytic electrodes.

Walt, D.R., **Fiber Optic Sensors, Apparatus and Detection Methods Using Fluid Erodible Controlled Release Polymers For Delivery of Reagent Formulation**, U.S. Patent Pending.

Walt, D.R., **Sensitivity Enhancement of pH Optrodes by Inner Filter Effects**, Analytical Chemistry, (in press).

Weaver, J.C., **Sampling: A Critical Problem in Biosensing**, Medical and Biological Engineering and Computation, 28, 1990, pp.B3-B9.

Biosensing is widely recognised to be of potentially major importance to medicine and related fields, but in spite of a large number of impressive and important advances, widespread practical application has lagged. We examine the thesis that 'sampling' is a process that involves all of the phenomena associated with the transport of analyte molecules to the active sensor site, and that problems associated with this process are now the limiting factor in further use of many existing biosensors. We conclude that an integrated process of sampling and sensing should be emphasized in developing new biosensing systems, and propose several new approaches.

Werme, C., Boehm, P., Cooke, M., Oberacker, D., Jacison, M. & Redford D., **Assessing Potential Effects of Incinerating Organic Wastes at Sea: Development and Field Testing of the Marine Biological Assessment Sampler**, Marine Pollution Bulletin, 19, 1988, pp.602-604.

Williams, G.B., Weaver, J.C. & Demain, A.L., **Rapid Microbial Detection and Enumeration Using Gel Microdroplets and Cloroimetric or Fluorescence Indicator Systems**, Journal of Clinical Microbiology, Vol. 28, No. 5, 1990, pp.1002-1008.

A new micromethod employing gel microdroplets (GMDs) and optical measurements can be used for rapid detection and enumeration of viable microorganisms (J.C. Weaver, G.B. Williams, A.M. Kilbanov and A.L. Deman, Bio/Technology 6:1084-1089, 1988) and has several potential applications in clinical microbiology. This method involves entrapping microorganisms in GMDs (10 to 100 um in diameter) which are surrounded by a hydrophobic (low dielectric) fluid, subsequently distinguishing occupied and unoccupied GMDs with colorimetric or fluorescence indicators, counting both occupied and unoccupied GMDs, and applying Poisson statistical analysis. Acid-producing microorganisms were used to compare colorimetric and fluorescence pH indicator systems. Fluorescence systems were generally superior, particularly for detection before microbial growth occurred.

BIOGRAPHIES OF PRESENTERS

Dr. Paul D. Boehm
Arthur D. Little, Inc., Marine Sciences Division

Dr. Boehm joined Arthur D. Little, Inc. as the Director of its Marine Sciences Division in 1989. In addition to managing the Marine Sciences Division, Dr. Boehm is currently serving as the Program Manager and Senior Advisor to Exxon on Exxon Valdez oil spill studies and is on the Scientific Review Board to the U.S. Minerals Management Service for the Bahia Las Minas oil spill in Panama. Prior to joining Arthur D. Little, Dr. Boehm was Technical Director at Battelle Ocean Sciences. While at Battelle, Dr. Boehm helped design, implement and manage the national marine monitoring program, "Mussel Watch Program" for the National Oceanic and Atmospheric Administration.

Dr. Boehm received his B.S. in chemical engineering from the University of Rochester and his M.S. and Ph.D. in chemical oceanography from the University of Rhode Island.

Professor Charles L. Cooney
MIT, Department of Chemical and Biochemical Engineering

Professor Cooney is currently the Associate Director of the MIT Biotechnology Process Engineering Center and is a Professor of Chemical and Biochemical Engineering at MIT. Professor Cooney currently serves on the editorial board of a variety of biotechnology and biochemistry publications and is editor of Biotechnology Advances. He also serves on the Board of Directors for Genzyme Corp., Pall Corp. and the Astra Research Center in India.

Professor Cooney received his B.S. in chemical engineering from the University of Pennsylvania and his S.M. and Ph.D. from MIT in biochemical engineering. He is a member of Sigma Xi and in 1989 received the gold medal from the Institute for Biotechnological Studies.

Dr. Nelson M. Frew
Woods Hole Oceanographic Institution, Chemistry Department

Dr. Frew first joined the Woods Hole Oceanographic Institution's (WHOI) Chemistry Department as a postdoctoral fellow in 1971. Since 1982 he has held the position of Senior Research Specialist at WHOI. Dr. Frew's present research interests are in the study of sources, composition and distribution of surface-active organic materials in the marine microlayer and their relationship to productivity and physical dynamics; effects of biological surfactants on gas exchange rates at the air-sea interface; and application of high resolution gas chromatography, supercritical fluid chromatography and chemical ionization mass spectrometry to characterization of marine organic matter.

Dr. Jean-Francois Hamel
MIT, Department of Chemical Engineering

Dr. Hamel is a Lecturer and a Research Engineer in the Chemical Engineering Department and the Biotechnology Process Engineering Center at MIT. His undergraduate degree, in chemical engineering was obtained at the University of Compiegne, France and his graduate work was completed in biochemical engineering at MIT. His major research interests include the isolation and purification of biological products, enzyme biocatalysis, scale-up fermentation/cell processes and integration of unit operations.

Dr. Irving John Higgins
Cranfield Institute of Technology, Cranfield Biotechnology Centre

Dr. Higgins currently serves as Director of the Cranfield Biotechnology Centre. He is also Director and Co-founder of Cranfield Biotechnology Ltd. and Euro-Laboratories Ltd. Prior to joining the Cranfield Institute of Technology, Dr. Higgins held posts at a variety of institutions including University of Kent, Howard Hughes Medical Institute, University of Miami and University of Sheffield. Dr. Higgins has also served as a consultant to a large number of industries with interests in biotechnology research. His research interests include bioelectronics, biosensors, biocatalysis, biodegradation, medical diagnostic technology and microbial hydrocarbon metabolism.

Dr. Higgins received his B.S. in biochemistry and Ph.D. in microbial biochemistry from Liverpool University. He is the author of several books on biotechnology including Biosensors published in 1987 by The Royal Society.

Professor Mark E. Meyerhoff
University of Michigan, Department of Chemistry

In 1979, Professor Meyerhoff joined the faculty of the University of Michigan, Department of Chemistry. He is currently conducting research in the areas of ion-selective electrodes, gas sensors, biosensors, bioanalytical chemistry, enzyme labelled competitive binding assays and ion-chromatography. Professor Meyerhoff is currently a member of the National Research Council Panel on New Measurement Technologies for the Ocean.

Professor Meyerhoff received his B.A. in chemistry from Herbert H. Lehman College and his Ph.D. in chemistry from the State University of New York at Buffalo.

Dr. John J. Stegeman
Woods Hole Oceanographic Institution, Department of Biology

Dr. Stegeman is a Senior Scientist and Watson Professor within the Department of Biology at the Woods Hole Oceanographic Institution. In addition to his appointment at WHOI, Dr. Stegeman also serves as a member of the Corporation of the Bermuda Biological Station. His primary research interests are in the functions and regulation of cytochrome P-450 forms in marine animals and the biology of environmental tumorigenesis in fish. Dr. Stegeman serves on a variety of advisory panels including the Natural Environmental Research Council (United Kingdom) on marine biotechnology and the NOAA Status and Trends Program.

Dr. Stegeman received his B.A. from St. Mary's College and his Ph.D. from Northwestern University.

Professor Gregory Stephanopoulos
MIT, Department of Chemical Engineering

Dr. Gregory Stephanopoulos is a Professor of Chemical Engineering at MIT. He received his B.S. degree from the National Technical University of Athens, M.S. degree from the University of Florida and Ph.D. degree from the University of Minnesota, all in chemical engineering. He joined, upon graduation in 1978, the Chemical Engineering faculty of the California Institute of Technology, where he served as Assistant and Associate Professor until 1985. In 1985 he was appointed Professor of Chemical Engineering at MIT where he has been ever since.

Professor Stephanopoulos' research interests span a broad spectrum of biotechnological applications. His current research focuses on the cultivation and physiology of mammalian cells, control and optimization of fermentation systems, amino acid and other organic acid fermentations, and growth and differentiation of wildtype and recombinant Bacillus strains for enzyme or recombinant fermentations. Professor Stephanopoulos' work has appeared in more than 90 publications and he has received 5 patents. He is presently the chairman of the Food, Pharmaceutical & Bioengineering Division of the American Institute of Chemical Engineers.

Dr. Craig D. Taylor
Woods Hole Oceanographic Institution, Department of Biology

Dr. Taylor joined the Woods Hole Oceanographic Institute in 1973 and is currently an Associate Scientist within the Department of Biology. Dr. Taylor's current research interests are quite varied and include: high resolution time-series studies of the cycling of carbon, nitrogen, sulfur and oxygen in coastal marine ecosystems; development and application of automated instrumentation for long-term *in situ* time-series analysis of inorganic nutrients, phytoplankton production and microbial activity in oceanic and coastal environments and; ecology and biogeochemistry of anaerobic microbial food chains.

Dr. Taylor received a B.S. and M.S. from Portland State University and an M.S. and Ph.D. from the University of Illinois (Urbana).

Professor David R. Walt
Tufts University, Department of Chemistry

Professor Walt is the Chairman of the Department of Chemistry at Tufts University. Some of Professor Walt's current research projects include: preparation of fiber optic sensors for clinical and environmental monitoring, preparation of bichromophoric polymers and enzyme-catalized organic synthesis. From July 1988 through December 1990, Professor Walt served on the Panel on CO_2 for the Ocean Studies Board of the National Research Council. Since June 1990, Professor Walt has been Chairman of the Panel on New Measurement Technologies for the Ocean-National Research Council.

Professor Walt received his B.S. in chemistry from the University of Michigan and his Ph.D. in chemistry and pharmacology from the State University of New York at Stony Brook.

Professor Daniel I.C. Wang
MIT, Department Chemical Engineering

Professor Wang is currently the Director of the MIT Biotechnology Process Engineering Center and a Chevron Professor of Chemical Engineering at MIT. Professor Wang's research interests include transport phenomena in animal cell bioreactors, bioreactor design in viscous fermentations, and biosensors in bioprocess monitoring and control. Professor Wang serves on the board of several biotechnology and biochemistry publications and on the advisory board of two research centers in China.

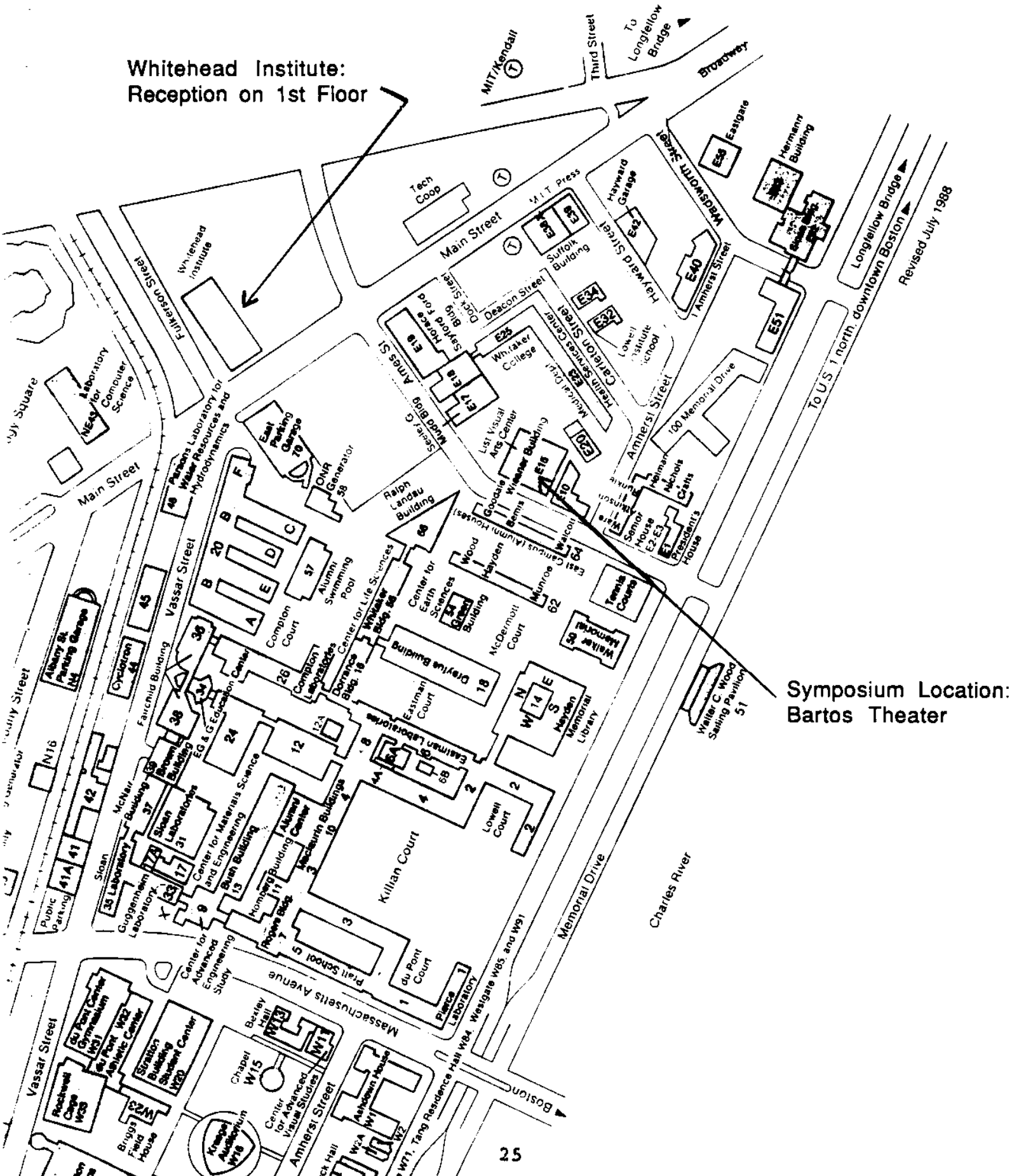
Professor Wang received his B.S. in chemical engineering and M.S. in biochemical engineering from MIT. He received his Ph.D. in chemical engineering from the University of Pennsylvania.

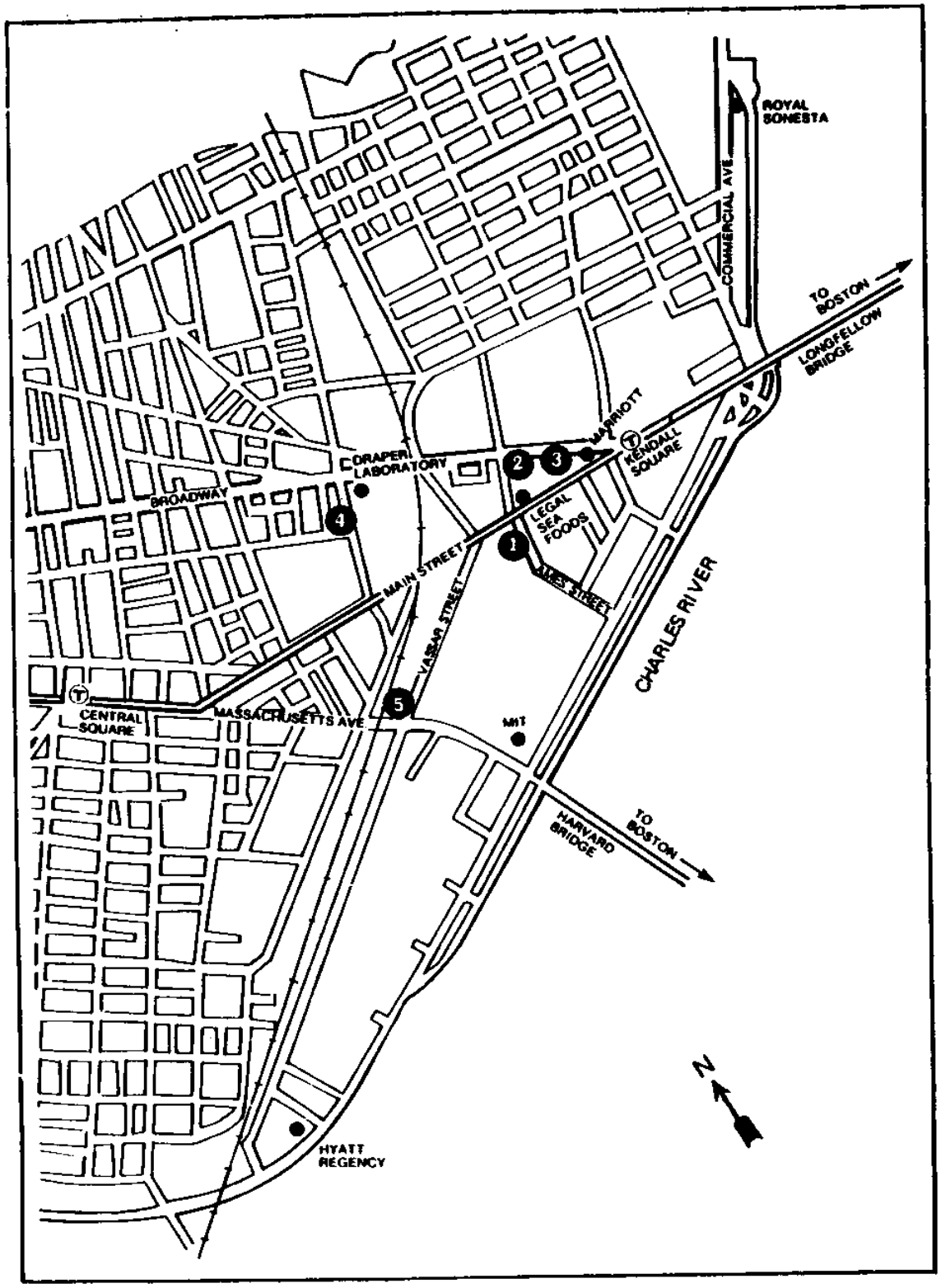
Dr. James C. Weaver
MIT, Harvard-MIT Division of Health Sciences and Technology

Dr. Weaver is the Associate Director of the Biomedical Engineering Center and Principal Research Scientist in the Harvard-MIT Division of Health Services and Technology. His primary interests are in biophysics as they relate to bioanalytical techniques and the effects of electromagnetic fields on living cells. Dr. Weaver's present areas of research include non-invasive sensing; electroporation mechanism and applications; interactions between biosensors and complex biological systems; and gel microdroplet technology and applications.

Dr. Weaver received his B.A. in physics from Carleton College and his M.S. and Ph.D. in physics from Yale University.

MAP of MIT CAMPUS





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